AUTOLOGOUS

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RECEIVER OPERATOR CHARACTERISTICS OF THE SYSMEX HPC MEASUREMENT USED TO INITIATE APHERESIS OF BLOOD HEMATOPOIETIC PROGENITOR CELLS

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Background: Hematopoietic progenitor cells (HPC) can be mobilized into the peripheral blood by the administration of G-CSF and/or GM-CSF and collected by apheresis for patients who are candidates for high dose chemotherapy and autologous stem cell transplantation. The initiation of apheresis is commonly based upon the number of CD34+ cells/μL in the blood, using a flow cytometric assay that typically requires 2 hours for sample preparation, acquisition, and reporting results. The availability of a new technology for rapidly measuring the content of hematopoietic progenitor cells in blood samples based upon size and impedance (Sysmex™) prompted an evaluation of how this method compares to the CD34+ flow cytometric assay. **Methods:** Prospective analysis was performed on 100 samples of cytokine mobilized peripheral blood from adult patients (ages 27-73) scheduled for collection of autologous HPC by apheresis. The HPC content was assayed using the Sysmex™ XE2100L (performed by the apheresis staff), and by the clinical lab using a dual platform BD FACScaliber™ and a modified ISHAGE protocol. The study population consisted of lymphoma (n = 32), multiple myeloma (n = 27), Hodgkin's disease (n = 9), and one patient each with CLL, germ cell cancer, and amyloidosis. **Results:** The mean number of CD34+ cells/μL determined using flow cytometry was 57.6/μL compared to 102.6/μL using the Sysmex™, with a correlation coefficient of 0.69. Of 71 patients being considered for apheresis, a mean number of 14.7 × 10^6CD34+ cells/kg were successfully collected from 62 patients (87%). An analysis of the receiver operating characteristics of the Sysmex assay, using the flow cytometric CD34+ cell assay as a "gold-standard," revealed that initiation of apheresis when the Sysmex HPC threshold was ≥31 cells/μL optimized the sensitivity and specificity of the test, and was the best predictor of when patients should begin apheresis collection (Table 1). The positive predictive value for a Sysmex™ HPC result of ≥31/μL was 80% (CD34+ cell counts of >20/μL); the negative predictive value for a Sysmex™ HPC of <31 cells/μL was 88% (12% had CD34+ cell counts of > 20/μL). **Conclusions:** Use of the Sysmex™ method for estimating HPC cell content of the peripheral blood is fast and reliable, with excellent sensitivity and specificity compared to flow cytometry. Using the Sysmex™ HPC result to initiate apheresis has reduced the average apheresis collection start time more than an hour (Table 1).

Table 1. *ROC of SysmexTM HPC Using CD34+ > 20/μL as the Standard*

HPC/μL	Sensitivity	Specificity
>10	97.3%	52.8%
>20	91.9%	69.8%
>30	83.8%	84.9%
>40	78.4%	88.7%
>50	67.6%	92.5%
>60	62.2%	94.3%
>70	59.5%	94.3%
>80	56.8%	94.3%

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HIGH DOSE THERAPY VERSUS ORAL MAINTENANCE: RESULT OF HD-CWS 96 STUDY FOR TREATMENT OF PATIENTS WITH METASTASIZED SOFT TISSUE SARCOMA (STS)

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Objectives: We studied the efficacy of high dose (HD) versus an oral maintenance treatment (OMT) in patients with STS stage IV. **Methods:** HD consisted of a tandem cycle of thiotepa (600 mg/m^2) + cyclophosphamide (4500 mg/m^2) and melphalan (120 mg/m^2) + etoposide (1800 mg/m^2). This treatment was optionally compared with each 4 OMT cycles consisting of trofosfamide (10 days 2*75 mg/m^2/d) + etoposide (10 days 2*25 mg/m^2/d) and trofosfamide (10 days 2*75 mg/m^2/d) + idarubicin (10 days 4*5 mg/m^2). Both groups were pretreated with the CEVAIE therapy (HD 7, OMT 9 cycles) consisting of carboplatin, etoposide, vincristine, actinomycin D, ifosfamide, and epirubicin. **Results:** Overall 753 patients were registered in CWS 96. From those 96 patients fulfilled study inclusion criteria (primary stage IV, <22 years, and intent to treat with study therapy). 45 were treated with HD, 51 with OMT. Whereas the study was not randomized, in the OMT and HD groups the main risk parameters were equally distributed. In the OMT group 15/51 (29%) fulfilled highest risk criteria (age ≥10 years and bone or bone marrow involvement), in the HD group 16/45 (35%) respectively. However, in the HD-group 11/45 were alive at a median observation time of 24.6 months (24.4%), in contrast to 26/51 OMT patients (50.9.%). Kaplan-Meier analysis demonstrates an overall survival for the whole group of 0.27 (OMT group: 0.31, HD group 0.20, log rank 0.0349). The proportional hazard analysis for patients with rhabdomyosarcoma only (77.1% of all patients) demonstrates an independent benefit of oral maintenance treatment on outcome. **Conclusions:** Oral maintenance therapy instead of tandem high dose therapy in patients with soft tissue sarcoma stage IV seems to be a promising option for patients with rhabdomyosarcoma.

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TARGETED RADIOTHERAPY IN THE CONDITIONING PRIOR TO HAEMATOPOIETIC STEM CELL TRANSPLANTATION: RESULTS OF A PHASE I TRIAL USING AN YTTRIUM-90-LABELLED ANTI-CD66 MURINE MONOCLONAL ANTIBODY DEMONSTRATING CONSISTENTLY HIGH BM UPTAKE

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We report the results of a phase I clinical trial using an yttrium-90 (Y-90) radiolabelled anti-CD66 IgG1 murine monoclonal antibody (TheraPharm GmbH) with conditioning therapy for patients receiving either an autologous or reduced intensity (RIC) allogeneic stem cell transplant (SCT) for myeloma or AML. This was a radiation dose escalation study with five patients at each radiation dose level of 5, 10, 25 and 37.5 MBq/kilogram lean body weight of recipient. Patients initially received indium-111-labelled anti-CD66 for biodistribution and dosimetry. **Patient Characteristics:** Ages 21-67 yrs (mean 56 yrs); 16 male, 4 female; disease indication for transplant: myeloma 18, poor risk AML 2. Autologous transplant 16; RIC-allogeneic 4. Patients received the therapeutic dose of radiation on day −14, for autologous SCT they also received melphalan 200 mg/m^2 on day −2; allogeneic SCT patients received a combination of fludarabine, melphalan and CAMPATH from day −8. **Results:** Excellent bone marrow targeting was seen in all patients with a 2-10 fold excess of radiation deliv-

ered to the bone marrow as compared with the liver. There was a linear relationship between the infused dose of Y-90 and the estimated radiation dose delivered to the BM. Mean absorbed radiation doses were: bone marrow 10.23 ± 1.8 cGy/MBq; liver 2.67 ± 2.0 cGy/MBq; spleen 7.10 ± 3.75 cGy/MBq. Total absorbed radiation doses at each Y-90 dose level are shown in Table 1. No additional toxicity due to the additional radiation was seen. Engraftment: neutrophils >0.5 by day +13.8 (range 11-22); platelets >50 by day +12.7 (range 10-22), no graft failures. In one patient with myeloma, focal uptake of radiolabelled antibody was seen at sites of disease activity suggesting in vivo targeting of myeloma. This is consistent with our finding of CD66 antigen expression by malignant plasma cells as shown by flow cytometry. There was a trend to greater disease response as the radiation dose increased, with a greater proportion of patients at the higher radiation dose levels achieving a CR. **Conclusions:** The radiolabelled anti-CD66 monoclonal antibody showed consistently excellent BM targeting and very low uptake by non-haematopoietic organs. Up to 30 Gy of radiation was delivered to the BM with no additional toxicity to other organs. Phase II studies are under way using the Y-90-labelled anti-CD66 in RIC-allogeneic SCT protocols and for autologous SCT for myeloma.

Table 1. *Organ Dosimetry*

Dose level (MBqkg)	Organ Dose in (Gy)		
	Bone Marrow	Liver	Spleen
5	4.1	1.4	1.1
10	9.1	1.3	2.4
25	15.6	3.7	12.6
37.5	25.0	7.4	5.1

GVH/GVL

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PREVENTION OF ACUTE GRAFT-VERSUS-HOST DISEASE DESPITE COMPENSATORY FUNCTION OF LYMPHOID ORGANS IN VIVO

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Acute graft-versus-host disease (aGVHD) results from alloreactive donor derived T cells attacking targets in the gastrointestinal tract, liver and skin. We observed the initiation and rapid kinetics of aGVHD in a murine model [FVB/N (H-2^q) into irradiated BALB/c (H-2^d)] using in vivo bioluminescence imaging. The transition from the initiation to the effector phase of aGVHD (day 3-4) was characterized by rapid T cell proliferation and upregulation of gut homing receptors $\alpha4\beta7$, $\alpha E\beta7$ and CCR9 on alloreactive T cells in Peyer's patches (PP), mesenteric lymph nodes (LN) and spleen, but not peripheral LNs. Therefore we asked whether the lack of specific lymphoid priming sites would lead to decreased alloreactive T cell infiltration in the gut compared to the liver and skin. Using PP deficient mice, we observed that mesenteric LN and spleen compensate for the lack of PP as alloreactive priming sites. Transplantation of PP and LN deficient mice ($LT\alpha^{-/-}$) showed that the spleen alone was sufficient to cause the complete profile of aGVHD with a time course similar to that of wild-type mice. Splenectomized mice with intact secondary lymphoid organs also developed aGVHD. Strikingly, treatment of splenectomized recipients with blocking antibodies against the lymphoid homing receptors L-selectin and MAdCAM-1 prevented GVHD with 100% survival (>120 d, $P < .0001$). Our study shows that multiple priming sites are involved in GVHD initiation, the spleen compensating for the lack of PP and mesenteric LN, and vice versa. In contrast, splenectomy and antibody blocking resulted in a clear survival benefit for all recipients. A.B. and S.S. contributed equally to this study.

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THE HAPLOIMMUNOSTORM SYNDROME: A DISTINCT CLINICAL ENTITY SEEN IN HLA-HAPLOIDENTICAL CELLULAR IMMUNOTHERAPY

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An immune-mediated anti-tumor response is the ultimate goal of allogeneic transplantation for relapsed, refractory malignancies. We developed a transplant protocol with less toxicity compared with standard allogeneic transplantation. We utilized multiple donor lymphocyte infusions after nonmyeloablative HLA-haploidentical stem cell transplantation for refractory disease. We have performed a total of 41 HLA-mismatched transplants with escalation of the CD3+ dose to 2×10^8 cells/kg using G-CSF primed product, with a conditioning regimen of 100 cGy total body irradiation. Our phase I/II study had 26 with hematologic malignancies. This therapy results in loss of detectable macrochimerism. Despite this, 13 responses, six major, occurred outside of macrochimerism. We have observed a new infusion related clinical entity named haploimmunostorm (HIS), observed after infusion. This syndrome occurred in 26 out of 30 (87%) patients with a CD3+ dose more than 1×10^8 cells/kg. In the syndrome, a constellation of symptoms occurred, some with variable penetrance, in which hyperpyrexia and malaise were a constant feature occurring as early as 4 hrs after cell infusion (median of 14 hrs). A morbilliform rash was seen in 40% of patients. Biopsies revealed no evidence of hyperacute or acute GVHD. Diarrhea was present in a 20% of patients; biopsies taken also failed to show any evidence of GVHD. Transient elevations of liver enzymes occurred in 40% of the patients usually. Steroids were used successfully if the HIS syndrome lasted more than 72 hrs. We used a Bioplex machine and analyzed 17 separate cytokine levels serially in these patients beginning with pre-treatment levels. Cytokine level analysis showed up to a 90 fold increase in baseline cytokine levels with significant increases of at least 10 fold in IFN-γ, IL-10, IL-13, IL-2, IL-5, IL-6, IL-7, IL-8, MCP-1, and MIP-1β. This syndrome appears to be immunologically based and represents neither hyperacute nor acute GVHD. This syndrome is different than an engraftment syndrome reported in some patients undergoing autologous transplant. Engraftment syndrome occurs at time of engraftment, opposed to HIS in which may be a rejection syndrome. Engraftment syndrome is similar to HIS but deviates with presence of capillary leak and pulmonary infiltrates. In summary, we have observed a new clinical entity that was not previously seen and is a result of the donors having a relatively intact immune system at the time of cell infusion.

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STATISTICAL MODELLING FOR CLINICAL AND GENETIC RISK FACTORS FOR GvHD AND SURVIVAL IN A COHORT OF EUROPEAN HLA MATCHED SIBLING TRANSPLANTS

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A cohort of 244 HLA matched sibling transplants from 5 centres within Europe were typed for SNPs or microsatellites (IL-1Rα, IL-4, IL-6, IL-10, IFNγ, TNFα, TNFR11, and ste-